(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 758 018 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
09.06.2004 Bulletin 2004/24

(51) Int Cl.[7]: **C12N 9/96**, A23L 1/00, C12N 9/16, C12N 9/24, C12N 9/42

(21) Application number: **96202071.5**

(22) Date of filing: **24.07.1996**

(54) **Salt-stabilized enzyme preparations**

Salz-stabilisierte Enzymzubereitung

Préparation enzymatique stabilisée avec du sel

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **28.07.1995 EP 95202066**

(43) Date of publication of application:
12.02.1997 Bulletin 1997/07

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EP 0 758 018 B1

Printed by Jouve, 75001 PARIS (FR)

Description

Field of the invention

[0001] The present invention relates to the conversion of enzyme solutions to solid enzyme preparations with improved drying yield, and storage and processing stability.

Background of the invention

[0002] The width of the application field and therefore the importance of enzymes in modern technology is increasing rapidly. One of the major drawbacks of these compounds however, is their aptitude to deactivation, caused not only by extremes of temperature, pH and the like during processing, but also occurring spontaneously on prolonged storage under otherwise normal conditions.

[0003] Often applied methods known in the art to diminish this problem include the use of a variety of additives claimed to stabilize enzymes in solution or the conversion of the enzyme solution to a dry formulation by means of freeze drying, spray drying or other techniques suitable for this purpose. The conversion of an enzyme in solution to a dry form is often obligatory when the application so demands (e.g. convenient mixing with other dry components).

[0004] Although drying in itself is a valuable tool in the improvement of the enzyme storage stability, the process step itself often causes a substantial loss of activity and the final product is still susceptible to inactivation. This activity loss during storage or processing is strongly dependent on moisture content of the preparation and this therefore has to be most stringently controlled to maintain the so valued product stability. This also includes a severe reduction of choice in the compounds suited for addition to the final enzyme product for the sake of standardization or other purposes.

[0005] In a variety of cases the dry enzyme is intended for applications in which the enzyme has to be incorporated in a product in which the moisture content can not be so strictly controlled. In these cases the enzyme stability is then severely reduced.

[0006] Several inventions have been made in the field of stabilizing enzymes against losses during drying and subsequent storage and handling.

[0007] The bulk of these inventions (such as presented in the patent (applications) US 3515642, EP 0501375A1 and WO 91/14773) are concerned with the addition of carbohydrate and, more specific, sugar or polyol components to the enzyme concentrate.

[0008] Also known in the art is the inclusion of components into the formulation with the aim to produce a glassy product at storage temperature, thus improving enzyme stability (EP 0383569A2).

[0009] Another approach is the addition to the formulation of one or several components able to bind moisture. This will reduce the water activity of the final preparation or temporarily prevent the interaction of water penetrating from the surroundings with the enzyme itself.

[0010] The use of organic and inorganic salts as a processing aid (e.g. to improve flowing behaviour of the product) or bulking/standardizing agent is well known. However, the use of inorganic salts to improve stability of dry enzyme preparations during processing or storage does not appear to be described in the art. In some cases enzyme destabilisation due to salt addition was even specifically mentioned (see e.g. Mikhailova et al. 1989, Vestsi Akad Navuk BSSR, Ser Biyal Navuk 6: 62-65).

[0011] EP 0522269A2 discloses the addition of insoluble calcium carbonate to an enzyme solution to be spraydried.

[0012] WO 92/1 1 347 discloses the incorporation of water soluble inorganic salts into enzyme containing granulates. The water soluble inorganic salts must be chosen such that they do not affect the storage stability of the enzyme granulate. WO 92/11347 provides several sodium- and potassium-salts as being suitable for this purpose. The water soluble inorganic salts are however added to the enzymes in dry form prior to their granulation by extrusion.

Description of the invention

[0013] The present invention provides a method for the production of solid enzyme formulations with improved drying yield and improved storage and processing stability of the resulting products. This is achieved by drying a solution comprising at least one enzyme and a water soluble inorganic salt, wherein the concentration of the inorganic salt is at least 5% (w/w) of the weight of the enzyme(s), and wherein the inorganic salt comprises a divalent cation selected from the group consisting of zinc and magnesium. More preferably the inorganic salt is zinc or magnesium sulphate. The solution comprising at least one enzyme and a water soluble inorganic salt may be prepared prior to drying. Also a combination of salts as well as a combination of enzymes can be used for this purpose. Zinc and Magnesium provide the best storage and processing stability. Sulphate is preferred as anion because it provides the best drying yield. The invention also provides solid enzyme formulations obtained by preparing a solution comprising at least one enzyme and a water soluble inorganic salt, wherein the concentration of the inorganic salt is at least 5% of the weight of the at

least one enzyme, and wherein the inorganic salt comprises a divalent cation selected from the group consisting of zinc and magnesium, more preferably an inorganic salt of zinc or magnesium sulphate, and drying the solution.

[0014] In the method of the invention, the salt is present while the enzyme is still in solution, i.e. prior to drying. Not only does this results in a higher yield during drying, but also the storage stability of the obtained dry enzyme preparations is improved as well as their processing stability. Processing stability is herein understood to mean the stability of the enzyme preparation during any handling of the enzyme preparation other than storage, such as e.g. the mixing of the enzyme preparation with other components or during the application of the enzyme.

[0015] Drying of the solution containing the enzyme and the salt will result in a solid composition which is homogeneous with respect to the distribution of the enzyme and the salt.

[0016] The drying of the enzyme and salt containing solution can be achieved by any drying method available to the skilled person, such as spray-drying, freeze drying, vacuum drying, fluid bed drying, and microwave drying. Drying of the enzyme-salt solution can also be combined with granulation methods which comprise e.g. the use of a fluid bed or a Multi-stage dryer (MSD). In case of the use of these granulation methods, the skilled person will understand that the obtained composition is not necessarily completely homogeneous. The obtained particles will usually consist of agglomerates of homogeneous particles or will consist of coated particles with a homogeneous core, with a homogeneous coating and/or combinations thereof. In any case there will be a homogenous interdispersion of the enzyme and the salt with respect to each other.

[0017] The specific examples of the invention demonstrate that the stabilising effect of the salt increases with increasing dosage of the salt to the enzyme solution, until at a certain point further increases in salt dosage no longer produce further improvement of the enzyme stability. For this reason at least 5%, preferably at least 15%, more preferably at least 30%, still more preferably at least 60%, and most preferably at least 90% (w/w) of salt is added to the enzyme solution, wherein the salt dosage is expressed as the weight percentage (w/w) of added salt based on the weight of the enzyme in solution, not including the weight of the crystal water of the salt crystals.

[0018] Although there is no upper limit to the addition of the salt from the view of the invention, a physical limit results from the maximum solubility of the salt in the enzyme concentrate. Higher salt/enzyme ratios can then only be realized by dilution of the enzyme solution or the use of a combination of salts.

[0019] Another point of consideration might be the dosage at which the enzyme is 'salted out', this being dependent on both the type of salt and the specific enzyme under consideration. Combinations of salts and enzymes giving rise to the 'salting out'-phenomenon are not excluded from the invention.

[0020] The invention can be used with enzymes or mixtures of enzymes from any type, including but not limited to phytases and other phosphatases, amylases, proteases, lipases and phospholipases, cellulases such as β-glucanases, pectinases, hemicellulases such as xylanases and other plant-cell wall degrading enzymes, esterases, rennets such as fungal proteases or chymosin, and β-galactosidases. It is to be understood that whenever referred to the enzyme or an enzyme, also mixtures of enzymes are included in these terms, irrespecitve of whether such mixtures are obtainable directly in a single fermentation or by mixing enzymes obtainable in different fermentions; and further including enzymes obtainable by fermentation of recombinant organisms.

The enzyme is preferably selected from the group comprising fungal phytases, fungal hemicellulases, fungal cellulases and bacterial proteases. More preferably the enzyme is selected from the group comprising phytases and endo-xylanases derivable from a fungus which belongs to the *Aspergillus niger* Group as defined by Raper and Fennell (1965, In: The Genus *Aspergillus*, The Williams & Wilkins Company, Baltimore, pp 293-344), β-glucanases and endo-xylanases derivable from a *Trichoderma* species, and proteases derivable from *Bacillus* species.

[0021] The invention also discloses solid compositions comprising at least one enzyme and a water soluble inorganic salt, wherein the dosage of the inorganic salt is at least 5% (w/w) of the weight of the enzyme(s) and the inorganic salt is a salt of a divalent cation selected from the group consisting of zinc and magnesium. More preferably the inorganic salt is zinc or magnesium sulphate. The dosage of the inorganic salt in the composition is at least 5% (w/w), preferably at least 15%, more preferably at least 30%, still more preferably at least 60% and most preferably at least 90% of the weight of the enzyme(s). Enzymes which may be prepared in compositions according to the invention include all those mentioned above, particularly those which are preferred.

[0022] In a further embodiment of the invention the above described solid compositions are used to prepare an animal feed. The preparation of animal feed often includes a pelleting step during which a significant amount of enzyme activity can be lost. The use of the solid composition of the invention reduces these losses in enzyme activity during pelleting. Typically an animal feed composition comprises raw materials of vegetable origin providing energy and metabolites for growth. The feed is supplemented with minerals and vitamins and it is common to add animal fat and animal proteins to the feed.

[0023] In yet another embodiment of the invention, a solid composition comprising a phytase with a improved storage stability is disclosed. The storage stability of the phytase in this solid composition is such that upon storage during 8 weeks, preferably in a closed container, at 30°C, less than 35%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the initial phytase activity is lost. The phytase compositions may comprise

an organic or inorganic physiologically acceptable carrier (e.g. grain-based carriers like wheat middlings, wheat flour, rice hulls, and so on, other organic carriers like tapioca, potato starch, cane sugar and so on, inorganic carriers like sodium chloride, potassium sulphate, silica and so on), in combination with phytase enzyme, possibly combined with other enzymes for functionalities' or conveniences' sake, and agents to further improve the products' appearance or functionality with respect to colour, flowing behaviour, physical, chemical or microbial stability, smell, taste, dust formation and so on. The skilled person will understand that similar compositions can be made for feed-enzymes other than phytase.

[0024] The following examples are provide by way of illustration of the invention.

Examples

Example 1

[0025] A fungal phytase concentrate (as produced by and obtainable from Gist-brocades) containing approximately 11% of enzyme by weight was split into two portions. To one portion 240 g/l of $MgSO_4.7H_2O$ was added. After dissolution of the salt, resulting in a clear solution, both solutions were spray dried using a Buchi lab scale spray drier with inlet temperature of 130°C and outlet temperature of 85°C.

[0026] The resulting powders were mixed with wheat middlings in a weight ratio of 1:10 to neutralize any differences in water activity between the preparations. After equilibration this was confirmed by measurement of the water activities, which both were found to be 0.45 at room temperature.

[0027] The final products were then stored at 30°C in closed jars and after storage the products were simultaneously analyzed for enzyme activity. After eight weeks of storage the activity loss of the preparation without salt addition amounted to 35%, against only 5% loss for the preparation with salt addition.

Example 2

[0028] Into separate portions of a concentrate of a Bacillus-derived alkaline protease (as produced by and obtainable from Genencor International Inc.) containing approximately 12% of enzyme protein by weight, 0 and 85 g/l of $MgSO_4.OH_2O$ was dissolved. These concentrates were then coated onto sodium sulphate carrier using a Niro STREA-1 lab scale fluid bed coater. The activity losses for this process step were 28% and 14% respectively.

[0029] The resulting particles were mixed with a surplus of bleach-containing commercial detergent powder for the European market and incubated in open jars at 35°C and 55% relative humidity. After 6 weeks of storage the remaining enzyme activity of the preparations was 42% with salt addition and only 30% without.

Example 3

[0030] 240 g/l of $MgSO_4.7H_2O$ was dissolved into a portion of Trichoderma-derived enzyme concentrate (as produced by and obtainable from Gist-brocades) with a total dry matter content of 25% by weight, containing both a β-glucanase and an endo-xylanase enzyme. The resulting solution and the original concentrate were separately coated onto a sodium sulphate carrier in a Glatt WSG-60 fluid bed coater. The process activity losses were 5% and 10% for the β-glucanase and 10% and 25% for the endo-xylanase with and without salt-addition respectively.

[0031] The resulting particles were incubated at 30°C and 62% relative humidity. After 12 weeks of storage the activity losses for the β-glucanase were 10% and undetectable and for the endo-xylanase the activity losses were 10% and 25%, for each enzyme with and without salt addition, respectively.

[0032] The granulates were also incorporated in a feed mixture and subjected to a pilot scale pelleting process at a temperature of 70°C. The β-glucanase activity losses amounted to 46% and 59% and the endo-xylanase losses were 37% and 66% with and without salt addition respectively.

[0033] The feed mixture used in the pelleting process was composed of:

Components	% (w/w)
Maize	50.00
Peas (22.9% raw protein)	3.50
Defatted soy (45.5% raw protein)	28.00
Tapioca	2.38
Animal meal (58% raw protein)	3.60
Fish meal (72.7% raw protein)	1.00

(continued)

Components	% (w/w)
Hydrolysed feather meal	1.00
Soy oil	1.75
Animal fat	3.50
Vitamin/mineral premix	3.15
Limestone	0.86
Monocalcium phosphate	0.96
Sodium chloride	0.30

Example 4

[0034] Different amounts of magnesium sulphate were dissolved in 100 ml of demineralized water after which 100 ml of a fungal phytase concentrate (as produced by and obtainable from Gist-brocades) containing app. 17% by weight of pure enzyme was added to each portion. After mixing the resulting solutions were spray-dried using a lab-scale Büchi 190 Mini Spray Dryer with inlet/outlet temperatures of 140 and 80°C respectively.

[0035] The resulting powders were mixed with wheat middlings in a ratio of 1:9. After equilibration water activity values at 35 °C were measured for control sample and sample with highest salt dosage to exclude water activity effects. The mixtures were subsequently incubated in closed bottles at 35°C for 8 weeks, after which activity losses were measured.

[0036] The results are presented in the following table:

$MgSO_4$-dosage [g/g enzyme]	Mixture Water Activity [-]	Storage losses after 8 weeks at 35°C
0	0.292	52%
0.31	-	37%
0.61	-	26%
1.19	-	15%
1.84	-	15%
2.38	0.303	17%

Example 5

[0037] According to the same protocol as mentioned in example 4 different salts in a dosage of 80 mmol per 100 ml of phytase concentrate have been used to produce stabilized enzyme powders. Mixing with wheat middlings and stability testing took place analogously to example 4.
The results are presented in the following table:

Salt type	Water Activity [-]	Spray Drying losses	Storage losses after 8 weeks at 35°C
None	0.29	6%	52%
magnesium sulphate	-	7%	15%
magnesium chloride	0.30	26%	43%
magnesium nitrate	0.31	32%	27%
zinc sulphate	-	5%	9%
zinc chloride	-	48%	5%
calcium chloride	-	40%	18%
calcium nitrate	-	44%	13%
sodium sulphate	-	11%	51%
potassium sulphate	-	17%	36%
ammonium sulphate	-	6%	46%

Example 6

[0038] Using the protocol described in Example 5, three phytase containing solutions were prepared containing either no added salt, magnesium sulphate or zinc sulphate. These solutions were subsequently dried using freeze drying instead of spray drying. The freeze drying was accomplished by freezing the mixed enzyme/salt solutions in a flask by submerging the flask in liquid nitrogen, after which high vacuum was applied to remove the water. The resulting dry preparations were crushed in a mortar and mixed with wheat middlings prior to stability testing for 8 weeks at 35°C in closed bottles. The results are presented in the following Table.

Salt type	Water activity of mixture	Activity losses after 8 weeks at 35°C.
None	0.31	46%
magnesium sulphate	0.31	16%
zinc sulphate	-	3%

Claims

1. A method for preparing a storage stable and processing stable solid enzyme composition comprising the step of drying a solution containing at least one enzyme and a water soluble inorganic salt, wherein the concentration of the inorganic salt is at least 5% (w/w) of the weight of the enzyme(s), and wherein the inorganic salt comprises a divalent cation selected from the group consisting of zinc and magnesium.

2. A method according to claim 1 comprising the steps of:

   a) preparing an aqueous solution comprising at least one enzyme and a water soluble inorganic salt, wherein the concentration of the inorganic salt is at least 5% of the weight of the enzyme(s), and wherein the inorganic salt comprises a divalent cation selected from the group consisting of zinc and magnesium; and
   b) b) drying the solution.

3. A method according to any one of claims 1-2, wherein the inorganic salt comprises a sulphate anion.

4. A method according to any one of claims 1-3, wherein the at least one enzyme is selected from the group consisting of a phytase, a protease, a hemicellulase and a mixture of a hemicellulase and a cellulase.

5. A method according to any one of claims 1-4, wherein the at least one enzyme is selected from the group consisting of a phytase from *Aspergillus,* a protease from *Bacillus*, a hemicellulase from *Aspergillus* and a mixture of a hemicellulase and a cellulase both from *Trichoderma*.

6. A method according to any one of claims 1-5, wherein the drying of the solution containing the at least one enzyme and the inorganic salt comprises the use of a dryer selected from the group consisting of a multistage dryer, a spray dryer, a fluid bed dryer and a freeze dryer.

7. A solid enzyme composition comprising (i) at least one enzyme and (ii) a water soluble inorganic salt, wherein the concentration of the inorganic salt is at least 5% (w/w) of the weight of the enzyme(s), and wherein the inorganic salt comprises a divalent cation selected from the group consisting of zinc and magnesium.

8. A solid enzyme composition according to claim 7, wherein the composition achieves homogeneity of the at least one enzyme and the inorganic salt by

   a) preparing a solution comprising at least one enzyme and a water soluble inorganic salt, wherein the concentration of the inorganic salt is at least 5% of the weight of the at least one enzyme, and
   b) drying the solution.

9. A solid enzyme composition according to claims 7 or 8, wherein the at least one enzyme is selected from the group consisting of a phytase, a protease, a hemicellulase, and a mixture of a hemicellulase and a cellulase.

10. A solid enzyme composition according to any one of claims 7-9, wherein the at least one enzyme is selected from

the group consisting of a phytase from *Aspergillus,* a protease from *Bacillus,* a hemicellulase from *Aspergillus* and a mixture of a hemicellulase and a cellulase both from *Trichoderma.*

11. A solid enzyme composition according to any of claims 7-10, wherein the inorganic salt comprises a sulphate anion.

12. A solid enzyme composition according to any one of claims 7-11, wherein the composition is homogeneous with respect to the distribution of the inorganic salt and the enzyme.

13. A solid enzyme composition according to any one of claims 7-12, wherein the composition is in the form of particles.

14. A solid enzyme composition according to claim 13, **characterized in that** the particles are coated.

15. A solid enzyme composition according to any one of claims 7-14 comprising a phytase that loses less than 35% of initial enzyme activity upon storage of the solid enzyme composition for 8 weeks at 30°C.

16. An animal feed comprising a solid composition as defined in any one of claims 7-15.

Patentansprüche

1. Verfahren zur Herstellung einer lagerstabilen und verarbeitungsstabilen festen Enzymzusammensetzung, bei dem man eine mindestens ein Enzym und ein wasserlösliches anorganisches Salz enthaltende Lösung trocknet, wobei die Konzentration des anorganischen Salzes mindestens 5 Gew.-%, bezogen auf das Gewicht des Enzyms bzw. der Enzyme, beträgt und das anorganische Salz ein zweiwertiges Kation aus der Gruppe bestehend aus Zink und Magnesium enthält.

2. Verfahren nach Anspruch 1, bei dem man:

a) eine mindestens ein Enzym und ein wasserlösliches anorganisches Salz enthaltende Lösung herstellt, wobei die Konzentration des anorganischen Salzes mindestens 5 Gew.-%, bezogen auf das Gewicht des Enzyms bzw. der Enzyme, beträgt und das anorganische Salz ein zweiwertiges Kation aus der Gruppe bestehend aus Zink und Magnesium enthält; und
b) die Lösung trocknet.

3. Verfahren nach Anspruch 1 oder 2, bei dem das anorganische Salz ein Sulfat-Anion enthält.

4. Verfahren nach einem der Ansprüche 1-3, bei dem das mindestens eine Enzym aus der Gruppe bestehend aus einer Phytase, einer Protease, einer Hemicellulase und einem Gemisch aus einer Hemicellulase und einer Cellulase stammt.

5. Verfahren nach einem der Ansprüche 1-4, bei dem das mindestens eine Enzym aus der Gruppe bestehend aus einer Phytase aus *Aspergillus,* einer Protease aus Bacillus, einer Hemicellulase aus *Aspergillus* und einem Gemisch aus einer Hemicellulase aus *Trichoderma* und einer Cellulase aus *Trichoderma* stammt.

6. Verfahren nach einem der Ansprüche 1-5, bei dem man beim Trocknen der das mindestens eine Enzym und das anorganische Salz enthaltenden Lösung einen Trockner aus der Gruppe bestehend aus einem Mehrstufentrockner, einem Wirbelschichttrockner und einem Gefriertrockner verwendet.

7. Feste Enzymzusammensetzung, die (i) mindestens ein Enzym und (ii) ein wasserlösliches anorganisches Salz enthält, wobei die Konzentration des anorganischen Salzes mindestens 5 Gew.-%, bezogen auf das Gewicht des Enzyms bzw. der Enzyme, beträgt und das anorganische Salz ein zweiwertiges Kation aus der Gruppe bestehend aus Zink und Magnesium enthält.

8. Feste Enzymzusammensetzung nach Anspruch 7, bei der die Homogenität des mindestens einen Enzyms und des anorganischen Salzes dadurch erreicht wird, daß man

a) eine mindestens ein Enzym und ein wasserlösliches anorganisches Salz enthaltende Lösung herstellt, wobei die Konzentration des anorganischen Salzes mindestens 5 Gew.-%, bezogen auf das Gewicht des

mindestens einen Enzyms, beträgt; und
b) die Lösung trocknet.

9. Feste'Enzymzusammensetzung nach Anspruch 7 oder 8, bei der das mindestens eine Enzym aus der Gruppe bestehend aus einer Phytase, einer Protease, einer Hemicellulase und einem Gemisch aus einer Hemicellulase und einer Cellulase stammt.

10. Feste Enzymzusammensetzung nach einem der Ansprüche 7-9, bei der das mindestens eine Enzym aus der Gruppe bestehend aus einer Phytase aus *Aspergillus*, einer Protease aus *Bacillus*, einer Hemicellulase aus *Aspergillus* und einem Gemisch aus einer Hemicellulase aus *Trichoderma* und einer Cellulase aus *Trichoderma* stammt.

11. Feste Enzymzusammensetzung nach einem der Ansprüche 7-10, bei der das anorganische Salz ein Sulfat-Anion enthält.

12. Feste Enzymzusammensetzung nach einem der Ansprüche 7-11, die hinsichtlich der Verteilung des anorganischen Salzes und des Enzyms homogen ist.

13. Feste Enzymzusammensetzung nach einem der Ansprüche 7-12 in Form von Teilchen.

14. Feste Enzymzusammensetzung nach Anspruch 13, **dadurch gekennzeichnet, daß** die Teilchen beschichtet sind.

15. Feste Enzymzusammensetzung nach einem der Ansprüche 7-14 mit einer Phytase, die bei Lagerung der festen Enzymzusammensetzung über einen Zeitraum von 8 Wochen bei 30°C weniger als 35% der anfänglichen Enzymaktivität verliert.

16. Tierfutter, das eine feste Zusammensetzung nach einem der Ansprüche 7-15 enthält.

Revendications

1. Procédé pour la préparation d'une composition d'enzymes solide, stable au stockage et stable vis-à-vis du traitement, comprenant l'étape de séchage d'une solution contenant au moins une enzyme et un sel inorganique hydrosoluble, tandis que la concentration du sel inorganique est d'au moins 5% (en poids/poids) du poids de la(des) enzyme(s), et tandis que le sel inorganique comporte un cation divalent choisi dans le groupe consistant en le zinc et le magnésium.

2. Procédé selon la revendication 1, comprenant les étapes de:

a) préparation d'une solution aqueuse comportant au moins une enzyme et un sel inorganique hydrosoluble, tandis que la concentration du sel inorganique est d'au moins 5% du poids de la(des) enzyme(s), et tandis que le sel inorganique comporte un cation divalent choisi dans le groupe consistant en le zinc et le magnésium; et
b) séchage de la solution.

3. Procédé selon l'une quelconque des revendications 1-2, dans lequel le sel inorganique comporte un anion sulfate.

4. Procédé selon l'une quelconque des revendications 1-3, dans lequel la au moins une enzyme est choisie dans le groupe consistant en une phytase, une protéase, une hémicellulase et un mélange d'une hémicellulase et d'une cellulase.

5. Procédé selon l'une quelconque des revendications 1-4, dans lequel la au moins une enzyme est choisie dans le groupe consistant en une phytase *d'Aspergillus*, une protéase de *Bacillus*, une hémicellulase *d'Aspergillus* et un mélange d'hémicellulase et de cellulase toutes deux de *Trichoderma*.

6. Procédé selon l'une quelconque des revendications 1-5, dans lequel le séchage de la solution contenant la au moins une enzyme et le sel inorganique comporte l'utilisation d'un sécheur choisi dans le groupe consistant en un sécheur multi-étages, un sécheur à pulvérisation, un sécheur à lit fluidisé et un appareil de lyophilisation.

7. Composition d'enzymes solide, comprenant (i) au moins une enzyme et (ii) un sel inorganique hydrosoluble, dans laquelle la concentration du sel inorganique est d'au moins 5% (en poids/poids) du poids de la(des) enzymes(s), et dans laquelle le sel inorganique comporte un cation divalent choisi dans le groupe consistant en le zinc et le magnésium.

8. Composition d'enzymes solide selon la revendication 7, dans laquelle la composition réalise l'homogénéité de la au moins une enzyme et du sel inorganique par

a) préparation d'une solution comportant au moins une enzyme et un sel inorganique hydrosoluble, dans laquelle la concentration du sel inorganique est d'au moins 5% du poids de la au moins une enzyme, et
b) séchage de la solution.

9. Composition d'enzymes solide selon les revendications 7 ou 8, dans laquelle la au moins une enzyme est choisie dans le groupe consistant en une phytase, une protéase, une hémicellulase, et un mélange d'une hémicellulase et d'une cellulase.

10. Composition d'enzymes solide selon l'une quelconque des revendications 7-9, dans laquelle la au moins une enzyme est choisie dans le groupe consistant en une phytase d'*Aspergillus*, une protéase de *Bacillus*, une hémicellulase d'*Aspergillus* et un mélange d'hémicellulase et de cellulase toutes deux de *Trichoderma*.

11. Composition d'enzymes solide selon l'une quelconque des revendications 7-10, dans laquelle le sel inorganique comporte un anion sulfate.

12. Composition d'enzymes solide selon l'une quelconque des revendications 7-11, dans laquelle la composition est homogène en ce qui concerne la distribution du sel inorganique et de l'enzyme.

13. Composition d'enzymes solide selon l'une quelconque des revendications 7-12, dans laquelle la composition est sous la forme de particules.

14. Composition d'enzymes solide selon la revendication 13, **caractérisée par le fait que** les particules sont revêtues.

15. Composition d'enzymes solide selon l'une quelconque des revendications 7-14, comportant une phytase qui perd moins de 35% de son activité enzymatique initiale lors du stockage de la composition d'enzymes solide pendant 8 semaines à 30°C.

16. Aliment pour animaux, comportant une composition solide telle que définie dans l'une quelconque des revendications 7-15.